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(54) Title: RAPAMYCIN DERIVATIVES

(57) Abstract

Rapamycin derivatives, processes for their production, pharmaceutical compositions containing them, and their use as antifungal agents, tumor cell growth-inhibiting agents and immunosuppressants.

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TITLE
RAPAMYCIN DERIVATIVES

BACKGROUND OF THE INVENTION

The present invention relates to a novel compound and derivatives thereof, to processes for their production, to pharmaceutical compositions containing them, and to their use as antifungal agents, tumor cell growth inhibiting agents and as immunosuppressants. This invention also relates to a biologically pure culture of a microorganism which has the identifying characteristics of ATCC 55367.

Rapamycin is a naturally occurring macrocyclic triene antibiotic which can be produced by culturing a producing organism in an aqueous nutrient medium. Rapamycin was first isolated as an extract of Streptomyces hygroscopicus and has been reported to have antifungal activity (British Patent 1436447). Subsequently rapamycin has been implicated as an immunosuppressant (Martel R.R. et al., Can J. Physiol. Pharmacol., 55, 48-51, 1977). There is a need for more agents of this type.

The present invention provides a novel, naturally occurring rapamycin derivative, i.e., 16-nor-rapamycin, as well as derivatives thereof. 16-nor-rapamycin has been found to have useful antifungal activity and also immunosuppressant properties, and is expected to have useful tumor cell growth-inhibiting activity.

SUMMARY OF THE INVENTION

This invention relates to 16-nor-rapamycin as well as derivatives thereof. The invention, in a second aspect, further provides a process for the production of 16-nor-rapamycin which comprises cultivating a 16-nor-rapamycin producing microorganism and subsequently isolating therefrom 16-nor-rapamycin or derivatives thereof. This invention also relates to the compound produced by such process.

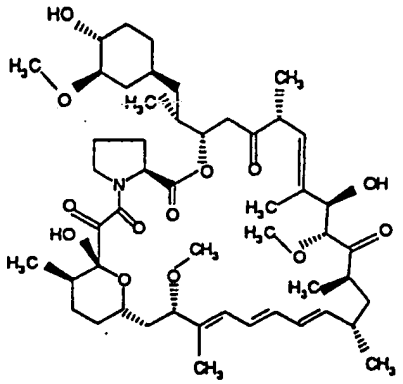
This invention also relates to a biologically pure culture of a microorganism which has the identifying characteristics of ATCC 55367 and active mutants thereof.

This invention further relates to a pharmaceutical composition comprising an effective, non-toxic amount of 16-nor-rapamycin or a derivative thereof, and a pharmaceutically acceptable carrier or diluent.

Further this invention relates to a method for treating fungal infection in an animal or human in need thereof which comprises administering to such animal or human an effective, non-toxic, anti-fungal amount of 16-nor-rapamycin or a derivative

thereof. In addition, this invention relates to a method for effecting immunomodulation in an animal or human in need thereof which comprises administering to such animal or human an effective, non-toxic, immunomodulatory amount of 16-nor-rapamycin or a derivative thereof. Futher, this invention relates to a method for inhibiting the growth of tumor cells sensitive to 16-nor-rapamycin in an animal or human in need thereof which comprises administering to such animal or human an effective, non-toxic, tumor cell growth inhibiting amount of 16-nor-rapamycin or a derivative thereof.

16-nor-rapamycin is believed to have the following structure:



The compound of the invention has the following characteristics:

i) apparent molecular weight of 899 by Electrospray Ionization (ESI) mass spectroscopy; and

ii) ^{1}H NMR spectroscopy consistent with the proposed structure.

16-nor-rapamycin may be obtained by the cultivation of a producing organism and the recovery of it or a derivative thereof from the culture. The term 'cultivation' (and derivatives of that term) as used herein means the deliberate aerobic growth of an organism in the presence of assimilable sources of carbon, nitrogen, sulphur and mineral salts. Such aerobic growth may take place in a solid or semi-solid nutritive medium, or in a liquid medium in which the nutrients are dissolved or suspended. The cultivation may take place on an aerobic surface or by submerged culture. The nutritive medium may be composed of complex nutrients or may be chemically defined.

It has been found that suitable microorganisms for use in the cultivation process according to the invention include bacterial strains belonging to the genus Streptomyces which are capable of elaborating 16-nor-rapamycin. One such producing organism was deposited on October 15, 1992 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A., in accordance with the provisions of the Budapest Treaty, and such organism was assigned ATCC accession number 55367.

It has been found that ATCC 55367, which was isolated from nature, and also mutants thereof, are capable of producing 16-nor-rapamycin. Thus, this invention also relates to a biologically pure culture of a microorganism which has the identifying characteristics of ATCC 55367, and mutants thereof.

The term 'mutant' as used herein includes any mutant strain derived from ATCC 55367 which arises spontaneously or through the effect of an external agent whether that agent is applied deliberately or otherwise and which retains the ability to produce 16-nor-rapamycin. Suitable methods of producing mutant strains including those outlined by H.I Adler in "Techniques for the Development of Microorganisms' in 'Radiation and Radioisotopes for Industrial Microorganisms', Proceedings of a Symposium, Vienna, 1973, page 241, International Atomic Energy Authority, and these include:

(i) Ionizing radiation (e.g. X-rays and g-rays), u.v. light plus a photosensitizing agent (e.g. 8-methoxypsoralen), nitrous acid, hydroxylamine, pyrimidine base analogues (e.g. 5-bromouracil), acridines, alkylating agents (e.g. mustard gas, ethyl-methane sulphonate), hydrogen peroxide, phenols, formaldehyde, heat, and

(ii) Genetic techniques, including, for example, recombination, transformation, transduction, lysogenisation, lysogenic conversion, protoplast fusion and selective techniques for spontaneous mutants.

The fermentation medium for cultivating ATCC 55367 suitably contains sources of assimilable carbon and assimilable nitrogen. Suitable sources of nitrogen include yeast extract, soyabean flour, and protein hydrolysates. Suitable carbon sources include glucose, and malt extract.

The cultivation may suitably be effected at a temperature of about 28°C, and the culture may suitably be harvested up to 7 days, advantageously about 3 to 4 days, after the initiation of fermentation in order to give an optimum yield of the desired product. This invention also relates to the product produced by such process.

The desired product or a derivative thereof may then be isolated from the culture medium and worked up and purified using conventional techniques for such compounds. All such isolation and purification procedures may conveniently be effected at cool to ambient temperature, for example at a temperature within the range of from 4 to 40°C, conveniently from 20 to 35°C.

The desired compound may readily be identified in a routine manner by testing for antifungal activity and/or by monitoring the h.p.l.c. retention time with photodiode array detector, or silica thin layer chromatography (TLC) R_F value.

Suitably, the separation procedure may include a high-performance liquid chromatography (HPLC) step, preferably as the last step. Elution may be effected using aqueous methanol or buffered aqueous acetonitrile.

16-nor-rapamycin and its derivatives may be crystalline or non-crystalline, and, if crystalline, may optionally be hydrated or solvated.

The 16-nor-rapamycin derivatives are preferably pharmaceutically acceptable derivatives. Derivatives may include salts with pharmaceutically acceptable counter ions.

The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight. An impure or less pure form of a compound according to the invention may, for example, be used in the preparation of a more pure form of the same compound or of a related compound (for example a corresponding derivative) suitable for pharmaceutical use.

This invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier or diluent and an effective amount of one or more compound of the invention

A compound of of the invention is administered in conventional dosage form prepared by combining a therapeutically effective amount of the compound ("active ingredient") with standard pharmaceutical carrier or diluents according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carrier are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl monostearate or glyceryl distearate along or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate and the like.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25 mg to about 1 g. If a liquid carrier is used, the preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable solution or suspension in an ampule or vial or nonaqueous liquid suspension.

To obtain a stable water soluble dosage form, a pharmaceutically acceptable salt of a compound of the invention is dissolved in an aqueous solution of an organic or inorganic acid, such as a 0.3M solution of succinic acid, or, preferably, citric acid. Alternatively, acidic derivatives can be dissolved in suitable basic solutions. If a soluble salt form is not available, the compound of the invention is dissolved in a suitable cosolvent or combinations thereof. Examples of such suitable cosolvents include, but are not limited to, alcohol, propylene glycol, polyethylene glycol 300, polysorbate 80, glycerin and the like in concentrations ranging from 0-60% of the total volume.

Tests indicate that the compound of this invention are useful in prophylactically or therapeutically inhibiting the growth of pathogenic fungi in a human or other animal in need thereof. The invention, therefore, includes methods of inhibiting the growth of pathogenic fungi in a human or other animal in need thereof which comprises administering to such human or animal an effective, non-toxic amount of a compound of of the invention.

By the term "pathogenic fungi" is meant fungi capable of producing disease in a human or other animal. Examples of pathogenic fungi include, but are not limited to *Candida albicans* and other candida species, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Asperqillus sp.* and *Sporotrichum sp.* Thus, the compounds may be used for the treatment of topical fungal infections in man caused by, among other organisms, species of Candida, Trichophyton, Microsporum or Epidermophyton or in mucosal infections caused by Candida albicans (e.g. thrush and vaginal candidiasis). They may also be used in the treatment of systemic fungal infections caused by, for example Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Coccidiodes, Paracocciciodes, Histoplasma or Blastomyces spp. They may also be of use in treating eumycotic mycetoma, chromoblastomycosis and phycomycosis. The ability of the compound of this invention to inhibit the growth of pathogenic fungi may be demonstrated or predicted by standard tests known and used for this purpose, for example, the yeast assay described hereinbelow.

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of compound would be for the purpose of inhibiting pathogenic fungi growth. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

Tests indicate that the compound of this invention are also useful for immunomodulation, i.e., inducing a suppression of a human's or animal's immune system. This invention, therefore relates, to a method of prophylactically or therapeutically inducing immunomodulation in a human or other animal in need thereof

which comprises administering an effective, non-toxic amount of such a compound of this invention to such human or other animal.

The ability of the compound of this invention to induce immunosuppression may be demonstrated in standard tests used for this purpose, for example, a mixed lymphocyte reaction test or a test measuring inhibition of T-cell proliferation measured by thimidine uptake.

The fact that the compound of this invention have utility in inducing immunosuppression means that they are useful in, *inter alia*, the treatment or prevention of resistance to or rejection of transplanted organs or tissues (e.g., kidney, heart, lung, bone marrow, skin, cornea, etc.); the treatment or prevention of autoimmune, inflammatory, proliferative and hyperproliferative diseases, and of cutaneous manifestations of immunologically mediated diseases (e.g., rheumatoid arthritis, lupus erythematosus, systemic lupus erythematosus, Hashimotos thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, nephrotic syndrome, psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitides, seborrheic dermatitis, Lichen planus, Pemplugus, bullous Pemphigold, Epidermolysis bullosa, uritcaris, angiodemas, vasculitides, erythemas, cutaneous eosinophilias, Alopecia areata, etc.); the treatment of reversible obstructive airways disease, intestinal inflammations and allergies (e.g., Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Chrohn's disease and ulcerative colitis) and food related allergies (e.g., migrane, rhinitis, and eczema), as well as multiple myeloma, hyperimmunoglobulin E, hepatitis B antigen negative chronic active hepatitis, Familial Mediterranean fever, Grave's disease, autoimmune hemolytic anemia, and primary biliary cirrhosis.

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of compound would be for the purpose of inducing immunosuppression. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The compound of this invention should also be useful for inhibiting the growth of tumor cells sensitive to a compound of the invention in an animal or human in need thereof. More specifically, the compounds should be useful for reducing tumor size, inhibiting tumor growth and/or prolonging the survival time of an animal or human bearing a tumor which is sensitive to a compound of this invention. Accordingly, this invention also relates to a method of inhibiting the growth of tumor cells sensitive to a compound of the invention in a human or other animal in need thereof comprising administering to such human or animal an effective, non-toxic amount of a compound of of the invention. One skilled in the art would be able, by routine experimentation, to

determine what an effective, non-toxic amount of compound would be for the purpose of treating carcinogenic tumors. Generally, however, an effective dosage is expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

The compound of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce such effect to a therapeutic or prophylactic degree. Such compound of the invention can be administered to such human or other animal in a conventional dosage form prepared by combining the compound of the invention with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

The route of administration of the compound of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous, intramuscular, subcutaneous, rectal, vaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

The daily parenteral and oral dosage regimens for employing compound of the invention to prophylactically or therapeutically inhibit the growth of pathogenic fungi, to prophylatically or therapeutically induce immunosuppression, or to therapeutically treat carcinogenic tumors will generally be in the range of about 0.05 to 100, but preferably about 0.5 to 10, milligrams per kilogram body weight per day.

The compound of the invention may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The preferred dosage amount of a compound of the invention to be employed is generally within the range of about 10 to 100 milligrams.

The compound of the invention may also be administered topically. By topical administration is meant non-systemic administration and includes the application of a compound of the invention externally to the epidermis, to the buccal cavity and instillation of such a compound into the ear, eye and nose, and where the compound does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of a compound of the invention (hereinafter referred to as the active ingredient) required for therapeutic or prophylactic effect on pathogenic fungi growth inhibition or

immunosuppression induction upon topical administration will, of course, vary with the compound chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician. A suitable topical dose of a compound of the invention will generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

While it is possible for an active ingredient to be administered alone as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g., from 1% to 2% by weight of the formulation although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

The topical formulations of the present invention, both for veterinary and for human medical use, comprise an active ingredient together with one or more acceptable carrier(s) therefore and optionally any other therapeutic ingredient(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and no deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required such as: liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 90-100 °C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an

agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or in organic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

No unacceptable toxicological effects are expected when the compound is administered in the above mentioned dosage ranges. However, it will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of the compound of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular animal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of the compound of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following Examples are, therefore, to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.

EXAMPLES

The following examples serve to illustrate the present invention.

A. PREPARATION OF 16-NOR-RAPAMYCIN

Example 1

Preparation of agar slant cultures: Agar slants prepared from 10ml of the slant medium [dextrose 10g/L; dextrin 40g/L; yeast extract 5g/L; NZ Amine Type A (Shefield) 2g/L;

soy peptone 20g/L; $CaCO_3$ 1g/L; agar 18g/L; in deionized water] were inoculated with approximately 0.1 ml of frozen culture stock, and incubated at 28°C for 5 to 10 days.
Preparation of seed culture: A 2L Ehrlenmyer flask containing 500 ml of seed medium [glucose 5g/L; dextrin 40g/L; soy peptone 20g/L; yeast extract 5g/L; NZ Amine Type A 2g/L; $CaCO_3$ 1g/L; in deionized water] was inoculated with the cells scraped from one agar slant. The flask was incubated at 28°C, and agitated at 250 rpm on a gyrotory shaker (1 inch throw). The seed was maintained under these conditions for 72 hours.
Inoculation and maintenance of production culture: A 15L fermentor containing 10L of sterile production medium [glucose 2g/L; malt extract 10g/L; yeast extract 10g/L; in deionized water] was inoculated with 300 to 500 ml of the seed culture. This culture was maintained at 28°C, 300 to 400 RPM agitation, 5 L/min. air, and a back pressure of 0.5 barr. The initial pH was 6.8 to 7.0, and was not controlled.
Harvest procedure: The fermentation broth was centrifuged at approximately 5000 x g for 40 minutes. The supernatant and cells were separated and delivered for purification.

Example 2

Preparation of agar slant cultures: Agar slant prepared from 10ml of the slant medium [dextrose 10g/L; dextrin 40g/L; yeast extract 5g/L; NZ Amine Type A (Shefield) 2g/L; soy peptone 20g/L; $CaCO_3$1g/L; agar 18g/L; in deionized water] were inoculated with approximately 0.1ml of frozen culture stock, and incubated at 28°C for 5 to 10 days.
Preparation of seed culture: 2L Ehrlenmyer flasks containing 500ml of seed medium [glucose 5g/L; dextrin 40g/L; soy peptone 20g/L; yeast extract 5g/L; NZ Amine Type A 2g/L; $CaCO_3$ 1g/L; in deionized water]were inoculated with the cells scraped from one agar slant. Each flask was incubated at 28°C, and agitated at 250 rpm on a gyrotory shaker (1 inch throw). The seed was maintained under these conditions for 72 hours.
Inoculation and maintenance of production culture: A 150L fermentor containing 100L of sterile production medium [glucose 2g/L; malt extract 10g/L; yeast extract 10g/L; in deionized water] was inoculated with 500 to 1500ml of the seed culture. This culture was maintained at 28°C, 200 RPM agitation, 75 L/min. air, and a back pressure of 0.5 barr. The initial pH was 6.8 to 7.0, and was not controlled.
Harvest procedure: The fermentation broth was centrifuged at approximately 5000 x g for 45 minutes. The supernatant and cells were separated and delivered for purification.

Isolation Procedure

At harvest whole broth (100L) was centrifuged in batches at 5000 x g for 45 minutes. The clarified broth was passed through a column of Diaion HP-20 (Mitsubishi Chemical

Industries LTD.)(4L). After washing with 4L of water, the column was eluted with 8L of acetone. The eluate was concentrated under reduced pressure and extracted with ethyl acetate followed by methylene chloride. The ethyl acetate and methylene chloride extracts were evaporated under reduced pressure and combined to give an oily residue and applied to a column of silica gel (250mL, E. Merck, Silica Gel 60, 70-230 mesh, Germany) packed in 80:20 hexane-acetone. The silica gel column was eluted sequentially with a step gradient of hexane in acetone (80:20; 60:40; 40:60 and 0:100). The hexane-acetone eluate (40:60) was concentrated under reduced pressure to give a crude residue. The crude residue was dissolved in methanol and injected onto a reversed phase column (E. Merck, Lobar Lichroprep, 40-63μm, 25x310mm) and eluted with a step gradient of methanol in water (60% to 100%) at a flow rate of 8mL/min and monitored at 280 nm. Active fractions were pooled according to HPLC analysis with photodiode array detector using Altex Ultrasphere ODS column (4.6x250mm, Beckman, USA) at a flow rate of 1mL/min using a mobile phase of 60:40 acetonitrile-ammonium acetate buffer (0.05M, pH 6.5). Fractions containing 16-nor-rapamycin were pooled and evaporated to dryness to give a crude residue. The crude residue was further purified using reversed phase semi-preparative HPLC (Altex Ultrasphere ODS column, 10x250mm, Beckman, USA) at a flow rate of 4 ml/min and eluted with a step gradient of 50 to 60% of acetonitrile in 0.05M ammonium acetate buffer (pH6.5). Fractions containing 16-nor-rapamycin were combined and desalted using reversed phase C_{18} Sep-Pak cartridge (Waters Associates, USA). The final purification step employed preparative TLC (E. Merck, Silica gel 60, Germany) using 70:15:15 cyclohexane-isopropanol-methylene chloride as eluent to yield 4 mg of 16-nor-rapamycin.

B. CHARACTERIZATION 16-NOR-RAPAMYCIN

NMR ($CDCl_3$, 360MHz): δ6.41 (dd,J=14.6, 10.4 Hz, 1H), 6.32 (dd,J=14.6, 10.0 Hz, 1H), 6.16 (dd,J=14.9, 10.0 Hz, 1H), 5.99 (d,J=10.4 Hz, 1H), 5.51 (dd,J=14.9, 9.1 Hz, 1H), 5.45 (s, 1H), 5.39 (d,J=10.0 Hz, 1H), 5.12 (ddd,J=7.0, 5.7, 3.0 Hz, 1H), 4.62 (dd,J=8.2, 4.6 Hz, 1H), 4.17 (d,J=7.0 Hz, 1H), 3.84-3.88 (m,1H), 3.78 (dd,J=10.3, 10.3 Hz, 1H), 3.63 (d,J=7.3 Hz, 1H), 3.54-3.60 (m, 1H), 3.56 (d, J=10.0, 5.5 Hz, 1H) 3.40 (s, 3H), 3.37 (s, 3H), 3.34-3.41 (m, 2H), 3.12 (s, 3H), 2.93 (ddd,J=10.9, 8.5, 4.2 Hz, 1H) 2.79-2.86 (m, 1H), 2.79 (dd, J= 170.2, 7.0Hz, 1H) 2.68 (dd, J= 17.0, 5.7 Hz, 1H) 2.21-2.32 (m, 1H), 1.88-2.18 (m, 4H), 1.77 (d, 0.95 Hz, 3H), 1.64 (s, 3H), 1.49-1.83 (m, 11H), 0.63-1.45 (m, 4H), 1.11 (d,J=6.7 Hz, 3H), 1.05 (d,J=6.6 Hz, 3H), 1.02 (d,J=6.7

Hz, 3H), 0.93 (d,J=6.0 Hz, 3H), 0.89 (d,J=6.2 Hz, 3H). Mass Spec (ESI^+/NH_4OAc) m/z 922 ($M+Na^+$), 917 ($M+NH4^+$), UV (MeOH)λmax 267, 277, 289nm.

Reversed phase HPLC comparison of rapamycin and 16-nor-rapamycin using an Altex Ultrasphere ODS column (4.6x250mm, Beckman, USA) at a flow rate of 1mL/min using a mobile phase of 60:40 acetonitrile-0.05M ammonium acetate buffer (pH 6.5) and monitored at 280nm gave retention time of 16.3 and 24.5 min respectively.

C. BIOLOGICAL ACTIVITY EXAMPLES

Compounds of the invention were analyzed for antifungal and immunosuppressive activity using the following assays.

Assay for Antifungal Activity

Yeast organism (*Saccharomyces cerevisiae*) in logarithmic growth were plated on complete agar medium (YPD). Compounds dissolved in an appropriate aqueous or organic solvent were placed in wells punched in the agar.

Plates were incubated for 48 hours and zones of inhibition were measured. The potency of compounds were quantified by regression analysis of plots of inhibition zone versus the log of drug concentration.

Mitogenesis Assay for Immunosuppressive Activity

Spleen cells from BDF1 female mice were established in RPMI with 10% fetal calf serum at 5 x 10^6/mL. One hundred mL aliquots of this suspension (5 x 10^5 cells) were dispensed into 96-well round-bottomed microtiter plates (Linbro, Flow Laboratories). Concanavalin A (5 mg/ml) was added as the mitogenic stimulus, and the final volume in the microtiter wells was adjusted to 200 mL with RPMI. Cell cultures were incubated for 72 hours at 37°C in a 5% CO_2 atmosphere and pulsed with 0.5 mCi ^3H-thymidine (specific activity 2.00 Ci/mole) for the last 18 hours of the 72 hour culture. The cells were harvested on an automated multiple sample harvester and cell-associated radioactivity counted in a Beckman liquid scintillation counter. The results are expressed as the mean values derived from quadruplicate measurements. Cell viability was determined by trypan blue exclusion after 72 hours of incubation. Compounds to be tested were added to the microtiter plates at the appropriate dilutions prior to the addition of cells.

16-nor- rapamycin showed IC_{12} of 2 ng/ml against Saccharomyces cerevisiae and IC_{50} of 6 nM in the mitogenesis assay. IC_{12} refers to the concentration of drug in the

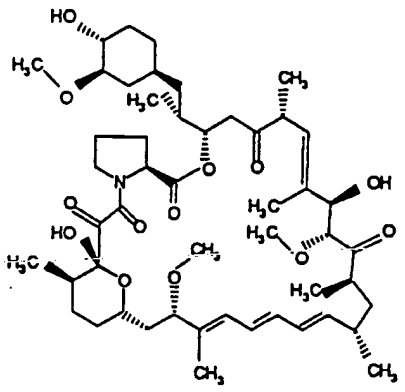
aforementioned antifungal agar diffusion assay where produces a 12 mm zone of inhibition. These results indicate that 16-nor-rapamycin has both antifungal and immunomodulatory activity.

While the above descriptions and Examples fully describe the invention and the preferred embodiments thereof, it is understood that the invention is not limited to the particular disclosed embodiments. Thus, the invention includes all embodiments coming within the scope of the following claims.

CLAIMS

What is claimed is:

1. A compound of the formula:



or a derivative thereof.

2. A biologically pure culture of a microorganism which has the identifying characteristics of ATCC 55367 or a mutant thereof.

3. A pharmaceutical composition which comprises an effective, non-toxic amount of the compound of Claim 1 and an inert, pharmaceutically acceptable carrier or diluent.

4. The composition of Claim 3 wherein the composition is in a dosage form adapted for parenteral administration.

5. The composition of Claim 3 wherein the composition is in a dosage form adapted for oral administration.

6. A method for treating fungal infection in an animal or human in need thereof, which comprises administering to such animal or human an effective, non-toxic, anti-fungal amount of the compound of Claim 1.

7. The method of Claim 6 wherein the amount of compound administered is from about 0.1 mg/kg to about 100 mg/kg of body weight.

8. A method of effecting immunomodulation in an animal or human in need thereof which comprises administering to such animal or human an effective, non-toxic, immunomodulatory amount of the compound of Claim 1.

9. The method of Claim 8 wherein the amount of compound administered is from about 0.1 mg/kg to about 100 mg/kg of body weight.

10. A method of inhibiting the growth of tumor cells which are sensitive to the compound of Claim 1 in an animal or human in need thereof which comprises

administering to such animal or human an effective, non-toxic, tumor cell growth-inhibiting amount of the compound of Claim 1.

11. The method of Claim 10 wherein the amount of compound administered is from about 0.1 mg/kg to about 100 mg/kg of body weight.

12. A process for the production of the compound of Claim 1 which comprises cultivating a microorganism which produces the compound of Claim 1, and subsequently isolating such compound from the culture.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10922

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 43/38; A61K, 31/40
US CL :514/ 411; 540/ 456; 435/253.5; 514/291; 435/119
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/ 411; 540/ 456; 435/253.5
514/291; 435/119

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,162,333 (FAILLI ET AL.) 10 NOVEMBER 1992.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 JANUARY 1994	Date of mailing of the international search report 28 FEB 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ROBERT T. BOND jd Telephone No. 703-308-1235

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